

CHAPTER 3SPECTROPHOTOMETRIC DETERMINATION OF AMMONIA IN INORGANIC
COMPOUNDS3.1 Introduction

Nitrogen constitutes 78.09% by volume of dry unpolluted air, but it is a minor element with respect to its abundance in the earth's crust. Atmospheric nitrogen forms an important raw material for the economical synthesis of bulk chemicals of industrial importance, viz. ammonia, urea, nitrate, nitrite etc. Therefore, estimation of ammonia and its derivatives assumes a great importance for chemical industry. Traditionally, ammonia has been estimated by direct titration with an acid. Kjeldahl method based on the conversion of nitrogen containing organic compounds to ammonium ion, is in use since 1883. The procedure is time proven for the determination of nitrogen in wide variety of chemical compounds. The various methods for the analysis of ammonia include titrimetric, spectrophotometric and chromatographic technique.

3.1.1 Titrimetric Determination of Ammonia(i) Acid-base titration

Ammonia is titrated directly with standard acid solution using methyl red as an indicator.⁸⁵ The end

point of the titration is also determined potentiometrically.⁸⁶ Ammonium ion in salts is titrated with standard sodium hydroxide solution conductometrically.⁸⁷⁻⁸⁹

Ammonia is treated with an excess of acid, which is then back titrated with standard alkali solution.⁹⁰

Ammonium ion reacts with the mercury-EDTA complex.

The hydrogen ion formed is titrated with standard alkali solution.⁹¹

(ii) Distillation Methods

The ammonium salt is treated with sodium hydroxide, followed by distillation of liberated ammonia into standard solution of hydrochloric or sulphuric acid and the excess of acid is back titrated with standard alkali solution.⁹² Only volatile basic substances such as pyridine and other amines interfere in the determination.

Alternatively, the liberated ammonia on treatment with alkali is absorbed in boric acid solution or in nickel ammonium sulphate solution. In the first case, the ammonium borate formed is titrated directly against standard acid solution using a mixture of methyl red-methylene blue as an indicator. In the second case,

nickel-amine complex formed is titrated against a standard acid solution. Both these methods require only one standard solution.⁹³

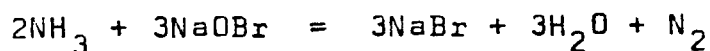
The ammonium salts are analysed by treatment of the sample directly with excess sodium hydroxide solution. The solution is boiled to remove ammonia, and the excess of alkali is titrated with standard acid solution.⁹² The method gives low results, mainly due to loss of alkali by spray and attack by hot alkali on the glass apparatus.

(iii) Formaldehyde Method

Ammonium salts react with formaldehyde to give hexamethylenetetramine and an acid. The later is titrated against standard alkali using phenolphthalein as an indicator.⁹³

(iv) Hypohalide Oxidation Procedures

Ammonium salts are determined by their decomposition with sodium hypobromite to nitrogen gas. However, the reaction is not considered to be quantitative.⁹⁴ Therefore, a correction factor should be used in the determination.



Addition of highly alkaline sodium hypobromite solution is found to give better results. The use of standard solution of potassium bromate as a source of bromine instead of potassium bromide is reported to give quantitative results. The unreacted hypobromite is determined iodometrically.⁹⁵

Arcand and Swift⁹⁶ utilized the hypobromite reaction as the basis of a coulometric titration. Bromine generated by the oxidation of bromide ion at platinum anode, is converted to hypobromite. The method has been useful to determine ammonia in low concentrations.

The use of calcium hypochlorite solution to generate hypobromite ion in situ by oxidation of potassium bromide has been exhaustively studied by Kolthoff and Stenger.⁹⁷ In the titration of ammonia in the presence of hypochlorite, the end point is detected by the reaction of bromine with Bordeaux dye. The determination is also carried out by adding excess of hypochlorite and the unreacted reagent is titrated with standard sodium thiosulphate solution. In another method, excess of hypochlorite is reduced with excess

sodium arsenite and the residual reducing agent is titrated with sodium hypochlorite solution. The above reactions are carried out in presence of bicarbonate or in alkaline medium. The pH of the reaction medium is critical. The loss of ammonia by volatilization and by partial oxidation to nitrous oxide increases with increase in pH. The optimum pH for the reaction is 8.2 to 8.5.

Laitinen and Woerner^{98,99} have titrated ammonia with hypochlorite in a bicarbonate medium containing bromide amperometrically.

A coulometric procedure is described by Liberti and Lazzari using hypohalite.¹⁰⁰

3.1.2 Gravimetric Methods

There are no specific quantitative gravimetric methods for the determination of ammonium ions. Ammonium ion is estimated as chloroplatinate.¹⁰¹ However, this method is not specific as almost all ions except chloride interfere in the procedure.

Ammonium tetraphenylborate obtained by the reaction of ammonium ion with tetraphenyl borate, is determined gravimetrically.¹⁰²

The thermogravimetric pyrolysis of the ammonium salt has been studied.¹⁰³ It was found that ammonium tetraphenylborate sublimes at 130°C. This observation forms the basis of quantitative determination of ammonia.

3.1.3 Spectrophotometric Method

Several colorimetric methods have been reported for the determination of ammonia or ammonium ions. Nessler's and Indophenol method are the most widely used procedures for determination of ammonia. Both the procedures are describe in detail in Chapter 4. Other methods are summarized briefly below :

Ammonia reacts with cupric ion to form cupric ammonium complex which is measured at 700 nm.¹⁰⁴ Ammonia is oxidized to nitrogen by hypobromite and excess of the hypobromite is determined by measuring absorbance at 330 nm.¹⁰⁵

Ammonia reacts with bispyrazolone in the presence of chloramine-T to form pink violet colored rubazoic acid.^{106,107} The reaction forms the basis of a spectrophotometric procedure for ammonia determination. Prochazkova¹⁰⁸ evaluated

critically the conditions of the reaction and proposed the mechanism of reaction. Ferrous ion, sulfate ion, cyanide ion, thiocynate ion, amines and amino acids are reported to interfere in the procedure.

Ammonia is chlorinated to trichloramine with excess of hypochlorite solution. The unreacted hypochlorite is destroyed with nitrite. The absorbance of reaction mixture after treatment with cadmium iodide-starch is measured at 615 nm.¹⁰⁹

On mixing with triphenyl phosphine-ruthenium solution, ammonia forms a complex which is extracted with benzene. The absorbance of the organic layer is measured at 620 nm.¹¹⁰

Other spectrophotometric procedures for the determination of ammonia involve the use of thymol and chloramine-T,¹¹¹ thymol and hypochlorite,¹¹² thymol and hypobromite,¹¹³ hypobromite,¹¹⁴ o-benzene-sulfamido-p-benzoquinone in dioxane¹¹⁵ or mercury (II) and methyl thymol blue reagent.¹¹⁶

3.1.4 Ammonia Sensing Electrode

In potentiometric estimation of ammonia, using gas sensing electrode based on internal pH probe are commonly

employed.¹¹⁷ Recently, several ammonium ion electrodes with better selectivity have been described¹¹⁸⁻¹²⁰ to determine ammonia in concentration range of 10^{-6} to 10^{-3} mole/litre. The determination should be carried out at constant temperature and with continuous stirring.¹²¹ The gas reacting with water, as well as volatile amines interfere in the determination.

3.1.5 Enzymatic Methods

In clinical laboratories, ammonia in blood is usually determined by the enzymatic method in which glutamate dehydrogenase catalyses the condensation of ammonia with α -ketoglutarate with simultaneous oxidation of the reduced nicotinamide adenine dinucleotide phosphate (NADPH). The decrease in absorbance at 340 nm is directly proportional to the ammonia concentration.¹²²

3.1.6 Miscellaneous Methods

The other methods for the determination of ammonia include atomic absorption spectroscopy,¹²³ molecular absorption spectroscopy,^{124,125} molecular emission spectroscopy,^{126,127} plasma emission spectroscopy,¹²⁸ polarography,^{129,130} high pressure liquid chromatography (HPLC),¹³¹ ion chromatography,^{132,133} and gasometric¹³⁴ techniques.

3.2 Field of Ammonia Determination

3.2.1 Pharmaceuticals

The aqueous ammonia solution is used in various pharmaceutical processes as a mild alkalizer. It is often preferred to the fixed alkalies because of its volatility. The excess of ammonia is readily detected by its odour and it can be easily removed by heat.

Ammonium salts commonly used in therapy include the carbonate, chloride and bromide. The carbonate liberates ammonia copiously which on inhalation, acts as a reflux stimulant. The bromide is used as a central depressant and the chloride as systemic acidifier. The latter enhances the diuresis of organic mercurials. Both the chloride and carbonate are the common ingredients in expectorant preparations. Ammonium acetate and ammonium citrate are also used in pharmaceutical formulations.

The determination of ammonia or ammonium ion involves back titration,⁹² formal titration⁹³ or titration of liberated ammonia from ammonium salt with the help of alkali.⁹²

Compounds like urea and saccharine are also determined as ammonia after their decomposition by treatment with alkali.¹³⁵ The ammonia formed is distilled off in standard acid solution which is then back titrated with standard alkali solution.

3.2.2 Fertilizers

Generally, ammonium sulphate, ammonium chloride, diammonium phosphate, ammonium nitrate or urea are used as nitrogen fertilizers.

The determination of ammonium nitrogen as such or in the presence of urea and nitrate requires different methods. AOAC¹³⁶ describes the formal titration method in case of ammonium nitrate and ammonium sulphate containing fertilizers. It also recommends a method involving distillation of ammonia in the presence of magnesium oxide. Ammonium ion in fertilizers is determined by indophenol method as free ammonium ion^{137,138} and in combination with urea and nitrate.¹³⁹

Ammonia selective membrane electrodes have been used to determine ammonia in fertilizers.¹⁴⁰

3.2.3 Biological Fluids

(i) Blood

In modern clinical laboratories, ammonia in blood is usually determined by an enzymatic method.¹²² After its separation by Selingson's modified diffusion technique, ammonia is determined by Nessler's reagent.^{141,142} In indophenol method, proteins and other materials are precipitated by treatment with ice cold 10% w/v of trichloroacetic acid solution in 1.3% w/v sodium hydroxide solution.¹⁴³ Serum ammonia is also estimated by Nessler's reagent.¹⁴⁴

(ii) Urine

Various nitrogen containing compounds are present in urine. They are in form of inorganic salts of ammonia as well as the organic nitrogenous compounds. Ammonia and urea are excreted in major proportion in urine.

For colorimetric analysis, ammonia is separated by microdiffusion or distillation prior to the color development in order to minimise the interference by other nitrogenous substances. Most widely used procedures are Nessler's method,¹⁴⁵ and indophenol method.^{146,147}

The ammonia formed by treatment of blood serum or urine with the enzyme (urease) is determined by Nessler's reagent. The color intensity of the reaction mixture is measured at 420 nm.¹⁴⁸ It is also determined using indophenol reaction.^{149,150}

3.2.4 Miscellaneous

Food materials like milk, fish, meat, etc. contain free ammonia. The concentration of ammonia increases on decomposition of fish and meat. For checking their quality, it is necessary to determine the free ammonia content of these food materials. Free ammonia is also present in water, and air. In all these cases, the colorimetric procedure of Nessler's reagent and indophenol method are employed to determine ammonia.

Determination of Ammonia and Ammonium Ion Using Hantzsch Reaction

Hantzsch reaction is mainly employed in the synthesis of pyridines. When ammonia reacts with acetylacetone and formaldehyde in aqueous media, yellow colored compound, 3,5-diacetyl-1,4-dihydrolutidine, is produced. Belman¹⁷ used this reaction for the detection of ammonia. However, the reaction is not investigated in details for the

quantitative determination of ammonia. It was, therefore, thought of interest to study the reaction conditions of Hantzsch reaction and to develop a new colorimetric method for determination of ammonia.

In the present work, various reaction conditions such as concentration of reactants, pH, time and temperature of reaction, etc. are standardized to obtain maximum color intensity. The method is based on formation of 3,5-diacetyl-1,4-dihydrolutidine under the reaction conditions.

Pure samples of ammonia, its salts and pharmaceutical formulations are analysed by proposed method. The method is applied to the determination of ammonia nitrogen in fertilizers. The results compare favourably with those obtained by official method.

3.3 Experimental

3.3.1 Apparatus

As described in Chapter 2.

3.3.2 Reagents and Materials

Ammonium Sulphate (BDH), Ammonium Chloride (BDH), Ammonium Bicarbonate (BDH), Diammonium Phosphate (SD'S), Ammonium Nitrate (BDH), Ammonium Acetate (BDH), Urea (BDH), Potassium Hydrogen Phthalate AR (BDH), Potassium Dihydrogen Phosphate AR (BDH), Potassium Chloride AR (BDH), Sodium Acetate (anhydrous) (BDH), Cupric Chloride (BDH), Formaldehyde (BDH), Hydrochloric Acid (BP), Acetic Acid (BDH), Acetyl-acetone (SD'S), Sodium Hydroxide (Pellets) (BDH), double distilled water were used in the study.

3.3.2.1 Preparation of Buffer Solution

The buffer solutions of pH 3.0 to 6.0 were prepared by mixing potassium hydrogen phthalate solution (0.2M) and hydrochloric acid solution (0.2M) or sodium hydroxide solution (0.2M).⁸¹

The buffer solution of pH 3.45 to 5.8 were prepared by mixing sodium acetate solution (0.2M) and acetic acid solution (0.2M).¹⁵¹

The final pH of buffer solutions was adjusted on pH meter.

3.3.2.2 Preparation of Reagent Solution

The reagent solution was prepared by mixing formaldehyde (150 ml) and freshly distilled acetylacetone (78 ml) with distilled water. The final volume was adjusted to 1 litre with water.

3.3.2.3 Preparation of Buffered Reagent Solution

The reagent solution was prepared by mixing acetate (16.4 g), acetic acid (12 ml), formaldehyde (150 ml) and freshly distilled acetylacetone (78 ml). The volume was adjusted to 1 litre with distilled water. The final pH of the solution was 4.7 ± 0.1 .

3.3.2.4 Preparation of Standard Ammonium Sulphate Solution

Ammonium sulphate (472.0 mg) (previously dried at 105°C for 2 hours) was dissolved in and diluted to 100 ml with water. The solution (5 ml) was diluted further to 100 ml with the same solvent. Final solution contained 50 mcg of nitrogen per ml of the solution.

3.3.2.5 Preparation of Standard Ammonium Chloride Solution

Ammonium chloride (382.0 mg) was dissolved in and diluted to 100 ml with water. The solution (5 ml) was diluted further to 100 ml with the same solvent.

3.3.2.6 Preparation of Standard Diammonium Phosphate Solution

Accurately weighed diammonium phosphate (471.4 mg) was dissolved in and diluted to 100 ml with water. The solution (5 ml) was diluted further to 100 ml with water.

3.3.2.7 Preparation of Standard Ammonium Nitrate Solution

Previously dried ammonium nitrate (571.4 mg) was dissolved in and diluted to 100 ml with water. The solution (5 ml) was diluted further to 100 ml with the same solvent.

3.3.3 Procedure

3.3.3.1 Determination of wavelength of maximum absorbance

Standard ammonium sulphate solution (2.0 ml) was pipetted into a 100-ml conical flask. Buffered reagent

solution (4 ml) was added to it. The reaction mixture was immersed in a boiling water bath for 15 minutes. It was cooled to room temperature. The content of flask was transferred quantitatively into a 25-ml volumetric flask with the help of water. The volume was adjusted to the mark with water. The absorbance of the colored solution was measured on Beckman Model 25 spectrophotometer in the range of 350 to 550 nm against blank.

The blank was prepared similarly in which volume of standard ammonium sulphate solution was replaced by equal volume of water.

Maximum absorbance of the colored solution was obtained at 412 nm (Fig.16).

3.3.3.2 Lambert-Beer's Curve for Ammonium Sulphate

Standard ammonium sulphate solution (0.25, 0.5, 1.0, 1.5, 2.0, 3.0 and 4.0 ml) was pipetted into a series of 100-ml conical flasks. Sufficient water was added to each flask to obtain 4ml of reaction mixture. The buffered reagent solution (4 ml) was added to it. The flasks were heated for 15 minutes in boiling water bath. After cooling to room temperature, the contents of the flask were transferred quantitatively to 25-ml volumetric flasks with the help of water and diluted to the mark with water. The absorbance of the colored product was measured at 412 nm against blank (Fig.17). The blank was prepared by replacing standard ammonium sulphate solution with equal volume of water.

3.3.3.3 Lambert-Beer's Curve for Ammonium Chloride, Diammonium Phosphate and Ammonium Nitrate

Standard solutions of ammonium chloride, diammonium phosphate or ammonium nitrate (0.25, 0.5, 1.0, 1.5, 2.0, 3.0 and 4.0 ml) were pipetted in to 100-ml conical flasks and analyzed as described under 3.3.3.2 (Fig.18,19,20).

3.3.4 Factors affecting the development of color

3.3.4.1 Effect of pH

Standard ammonium sulphate solution (2.0 ml) was treated with buffer solutions (phthalate or acetate buffer) of varying pH (3.0 to 6.0; 4 ml) and the non-buffered reagent (4 ml). The reaction mixture was immersed in a boiling water bath for 15 minutes. The mixture was cooled to room temperature and transferred quantitatively to a 25-ml volumetric flask. It was diluted to the mark with water. The absorbance of the colored solution was measured at 412 nm against blank prepared by replacing ammonium sulphate solution with equal volume of water.

Maximum absorbance was observed at pH 4.4 to 5.0 (Fig.21).

3.3.4.2 Effect of acetate buffer concentration

Different volumes of the acetate buffer solution (pH 4.7; 1 to 7 ml) were pipetted into a series of 100-ml conical flasks containing standard ammonium sulphate solution (2.0 ml). Non-buffered reagent solution (4 ml) was added to each flask and analyzed as described under 3.3.3.1. The absorbance was measured at 412 nm against blank.

Maximum absorbance was observed in presence of 3 ml buffer solution which remained constant on increasing buffer concentration (Fig.22). In the present work, 4 ml buffer solution was used.

3.3.4.3 Effect of reagent concentration

The absorbance at 412 nm of the colored products formed by the reaction of standard ammonium sulphate solution (2.0 ml) and buffer solution (pH 4.7; 4 ml) with different volume of non-buffered reagent solution increased with the increase in the concentration of the non-buffered reagent solution (Fig.23).

Maximum color intensity was obtained in the presence of 4 ml of the reagent solution which remained constant with further increase in the volume of reagent solution.

3.3.4.4 Effect of Temperature

The reaction of standard ammonium sulphate solution (2.0 ml) in presence of acetate buffer (pH 4.7; 4 ml) with non-buffered reagent (4 ml) was carried out at different temperatures (25°, 30°, 53° and 100°C) for 30 minutes. Absorbance of the reaction mixtures was measured at 412 nm (Fig.24).

Maximum color intensity was obtained at 100°C.

3.3.4.5 Effect of time of reaction

The reaction of standard ammonium sulphate solution (2.0 ml) with non-buffered reagent solution (4 ml) in the presence of acetate buffer (pH 4.7; 4 ml) was carried out in boiling water bath for different time intervals and analyzed as described under 3.3.3.1. Absorbance of the reaction mixture was measured at 412 nm against blank.

Maximum color intensity was obtained after 15 minutes (Fig.25) which remained constant on further heating.

3.3.4.6 Stability of colored product

Standard ammonium sulphate solution (2.0 ml) was reacted as described under 3.3.3.1 to form a yellow color product. One set of reaction mixture was stored in dark in stoppered bottle and another set was allowed to stand in diffuse sunlight. After different time intervals of storage, the color intensity was measured at 412 nm (Fig.26).

The absorbance of the reaction mixture stored in dark was constant for more than 24 hours, while that of the reaction mixture kept in diffuse sunlight was constant upto 4 hours.

3.3.5.1 Synthesis of 3,5-diacetyl-1,4-dihydrolutidine

Ammonium acetate (7.7g; 0.1M) was dissolved in water (200 ml) in a round bottom flask (500 ml). Acetylacetone (20g; 0.2M) and formaldehyde (7.5ml; 40%) were added to it. The reaction mixture was boiled immediately for 5 minutes with continuous stirring. It was allowed to cool to room temperature. The yellow solid separated was filtered. The residue was washed with water and dried. On recrystallization from ethanol, the product melted at 196-199°C. (reported¹² M.P. 198°C).

Analysis

Molecular Formula	:	$C_{11}H_{15}NO_2$
% Calculated for N	:	7.25
% Found	:	7.24

3.3.6.1 Analysis of Ammonium Sulphate

The powdered ammonium sulphate (ca. 472mg) was weighed accurately and dissolved in water and diluted to 100 ml with the same solvent. The solution (5 ml) was diluted further to 100 ml with water and analysed as described under 3.3.3.1 (Table IV).

3.3.6.2 Analysis of Ammonium Chloride

Ammonium chloride (ca.382.0 mg) was weighed accurately and dissolved in and diluted to 100 ml with water. The solution (5 ml) was transferred to 100-ml volumetric flask and diluted upto the mark with same solvent. Aliquot (2.0 ml) of the solution was analyzed as described under 3.3.3.1. The amount of ammonium chloride was determined by referring to the standard curve (Table V).

3.3.6.3 Analysis of Ammonium Bicarbonate

Ammonium bicarbonate (ca.564.7mg) was dissolved in and diluted to 100 ml with water. The solution (5 ml) was diluted further to 100 ml with the same solvent and analysis was carried out as described under 3.3.3.1 (Table V).

3.3.6.4 Analysis of Diammonium Phosphate

The powdered diammonium phosphate (ca.472 mg) was weighed accurately and dissolved in and diluted to 100ml with water. The solution (5 ml) was diluted further to 100 ml with water and analyzed as described under 3.3.3.1 (Table V).

3.3.6.5 Analysis of Ammonium Nitrate

Ammonium nitrate (ca.571.4mg) was weighed accurately and dissolved in and diluted to 100 ml with water. The solution (5 ml) was diluted further to 100 ml with the same solvent and analyzed as described under 3.3.3.1 (Table V).

3.3.6.6 Analysis of Ammonium Acetate

Ammonium acetate (ca.550.6mg) was weighed accurately and dissolved in and diluted to 100 ml with water. The solution (5 ml) was diluted further to 100 ml with same solvent and its analysis was carried out as described under 3.3.3.1 (Table V).

3.3.6.7 Analysis of Strong Solution of Ammonia

The strong solution of ammonia (ca.400 mg) was weighed accurately into a 100-ml volumetric flask containing 10 ml of water and diluted to mark with water. The solution (5 ml) was further diluted to 100 ml with the same solvent. The dilute solution (20ml) was analyzed as described under 3.3.3.1 (Table VI).

3.3.6.8 Analysis of Dilute Solution of Ammonia

The dilute solution of ammonia (ca.1.2 g) was weighed accurately and dissolved in and diluted to 100 ml with water. The solution (5 ml) was further diluted to 100 ml with the same solvent and the dilute solution (2.0ml) was analyzed as described under 3.3.3.1 (Table VI).

3.3.6.9 Analysis of Strong Solution of Ammonium Acetate

The strong solution of ammonium acetate (0.1 ml) was diluted to 100 ml with water. The solution (5 ml) was diluted further to 100 ml and the dilute solution (2.0ml) was analyzed as described under 3.3.3.1 (Table VI).

3.3.6.10 Analysis of Ichthammol

Ichthammol (ca.300 mg) was weighed and dissolved in water (5 ml). Copper chloride solution (2%; 2 ml) was added to it with constant stirring, and was kept aside for 20 minutes. The mixture was filtered through Whatman filter and washed thoroughly with water. The filtrate and washing were combined and diluted to 100 ml with the same solvent. The solution (2.0 ml) was analyzed as described under 3.3.3.1 (Table VI).

3.3.6.11 Analysis of ammonium nitrogen in the presence
urea in synthetic mixture

The synthetic mixtures of diammonium phosphate and urea were prepared (Table VII). The mixture equivalent to ammonium nitrogen (100 mg) was weighed accurately and dissolve in water and diluted to 100 ml. An aliquot (5 ml) of the solution was diluted further to 100 ml with water and the solution (2.0 ml) was analyzed as described under 3.3.3.1 (Table VII).

3.4 Results and Discussion

Ammonia has been determined spectrophotometrically using various methods. In most of the methods, the reaction is carried out in alkaline media. Highly alkaline medium causes loss of ammonia from the solution if the reagents are not added immediately. They are time bound and required fix order of reagent addition with many steps. The presence of some other ions interferes in the analysis. Therefore, it was desirable to establish a simple, accurate and precise method for determination of ammonia as well as ammonium ion.

Belman¹⁹ detected ammonia fluorimetrically using Hantzsch reaction. In the present work, the reaction conditions are standardized to estimate ammonia colorimetrically.

In the proposed method, ammonia was reacted with acetylacetone - formaldehyde reagent (4 ml) prepared in acetate buffer (pH 4.7), in boiling water bath. The yellow colored product formed showed maximum absorbance at 412 nm (Fig.16). The maximum color intensity was obtained after keeping the reaction mixture in boiling water bath for 15 minutes (Fig.25). The color remains stable for 4 hr

in diffuse day light and for more than 24 hours, if stored in a stoppered bottle in dark (Fig.26). Maximum color intensity was observed when the reaction carried out in a buffer of pH 4.4 to 5.0 (Fig.21). The Lambert-Beer's law is obeyed in the concentration range of 0.5 to 8 mcg of ammonium nitrogen per ml. of the reaction mixture (Fig.17). The optimum concentration range for the determination as evaluated from the Ringbom plot⁸³ was found to be 1 to 6 ppm of ammonia nitrogen. The effective molar absorptivity in terms of ammonia was found to be $1.7 \times 10^{-3} \text{ L mol}^{-1} \text{ cm}^{-1}$ or $1.4 \times 10^3 \text{ L mol}^{-1} \text{ cm}^{-1}$ in terms of ammonia nitrogen and the photometric sensitivity⁸⁴ of the color reaction was found to be 0.01 mcg of ammonia nitrogen cm^{-2} at 412 nm. The standard and percentage relative standard deviations were found to be ± 0.713 and 0.715% , respectively (Table IV). These results indicate that the method is precise and gives reproducible results.

Pure samples of pharmaceutical ammonium salts and its official formulations, as well as Strong and Dilute Solution of Ammonia were analysed by the proposed method and compared with official method^{136,152} (Table V,VI). The results by both the methods are in good agreement.

There is no approved AOAC procedure¹³⁷ for the determination of ammoniacal nitrogen in urea-ammonium phosphate based fertilizers. In the preliminary experiment, it was found that urea does not interfere in the determination of ammonia nitrogen by proposed procedure. Various synthetic mixtures containing urea with diammonium phosphate were analyzed by the proposed method. The satisfactory recovery of DAP was obtained (Table VII).

Ichthammol contains ammonia in form of sulphate as well as in form of sulphonate. Pharmacopoeial ichthammol is assayed directly by the proposed procedure after the precipitation of resinous matter with copper chloride solution. However, the commercial samples do not give clear solution after precipitation and hence they could not be analyzed by the proposed procedure. They are analyzed satisfactorily by USP¹⁵³ procedure.

In order to study the nature of the chromophore, 3,5-diacetyl-1,4-dihydrolutidine was synthesized by reacting ammonium acetate with acetylacetone and formaldehyde in aqueous medium. The product was dissolved in aqueous ethanol and scanned in the range of 350 to 550 nm (Fig.27). The reaction mixture containing ammonium

sulphate solution (20ml) and buffered reagent solution (4 ml) was also scanned between 350 to 550 nm. It shows similar characteristic of 3,5-diacetyl-1,4-dihydrolutidine with wave length of maximum absorbance at 412 nm (Fig.27).

It is evident from the above observation that 3,5-diacetyl-1,4-dihydrolutidine formed in the reaction mixture is responsible for the color.

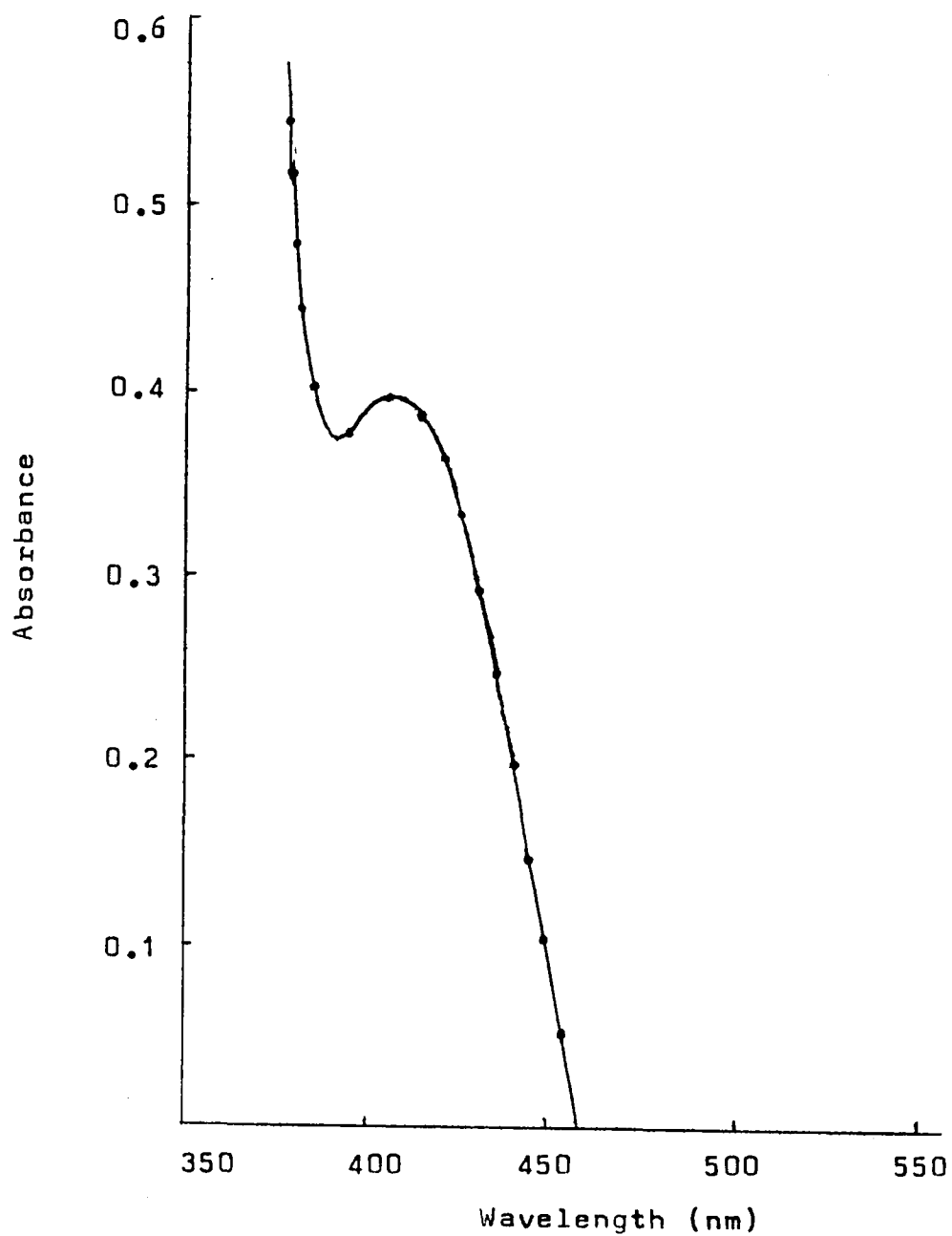


Figure 16 : Visible spectrum of the colored product obtained on reacting Ammonium Sulphate with reagent.

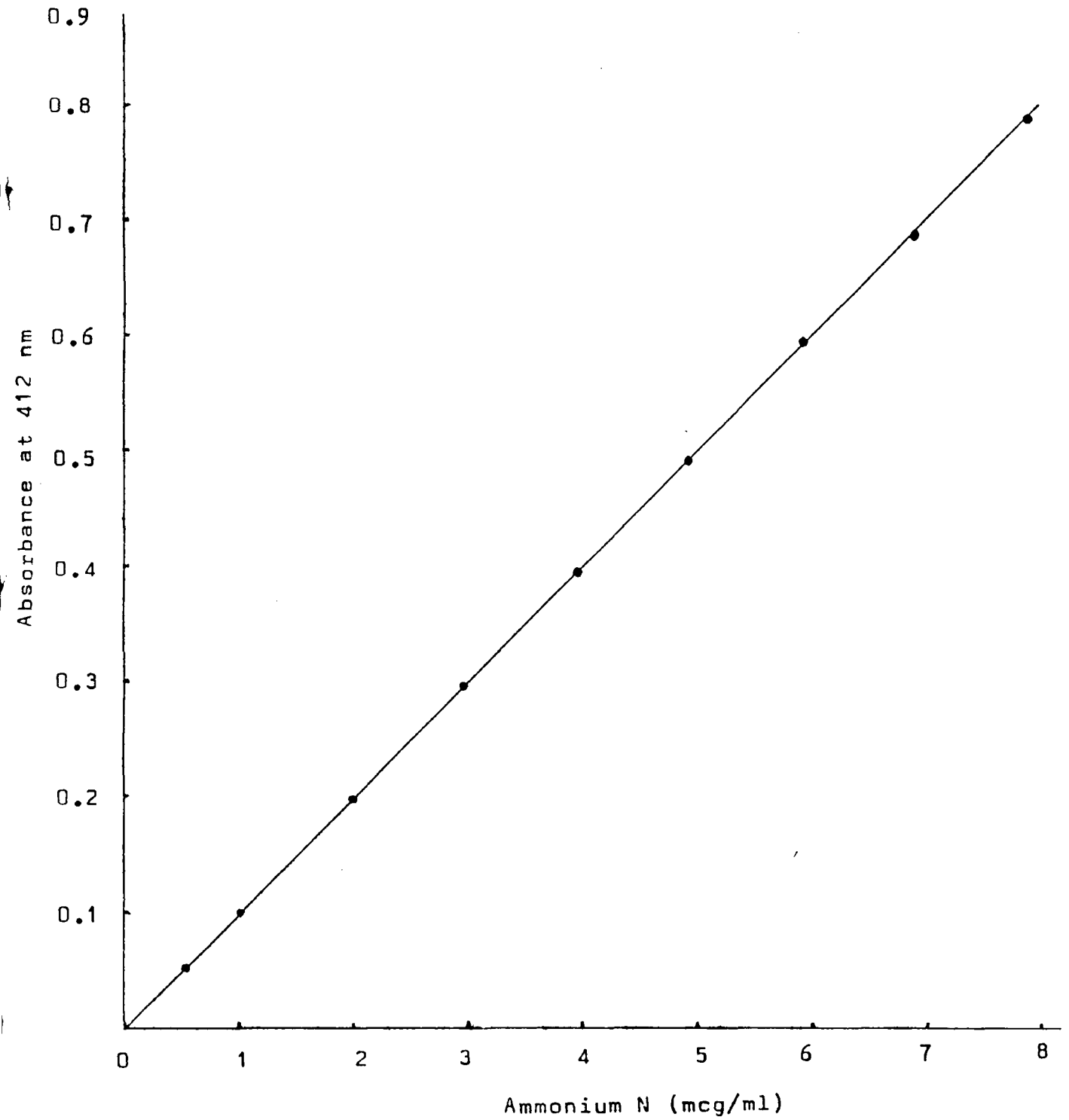


Figure 17 : Lambert-Beer's curve for Ammonium Sulphate.

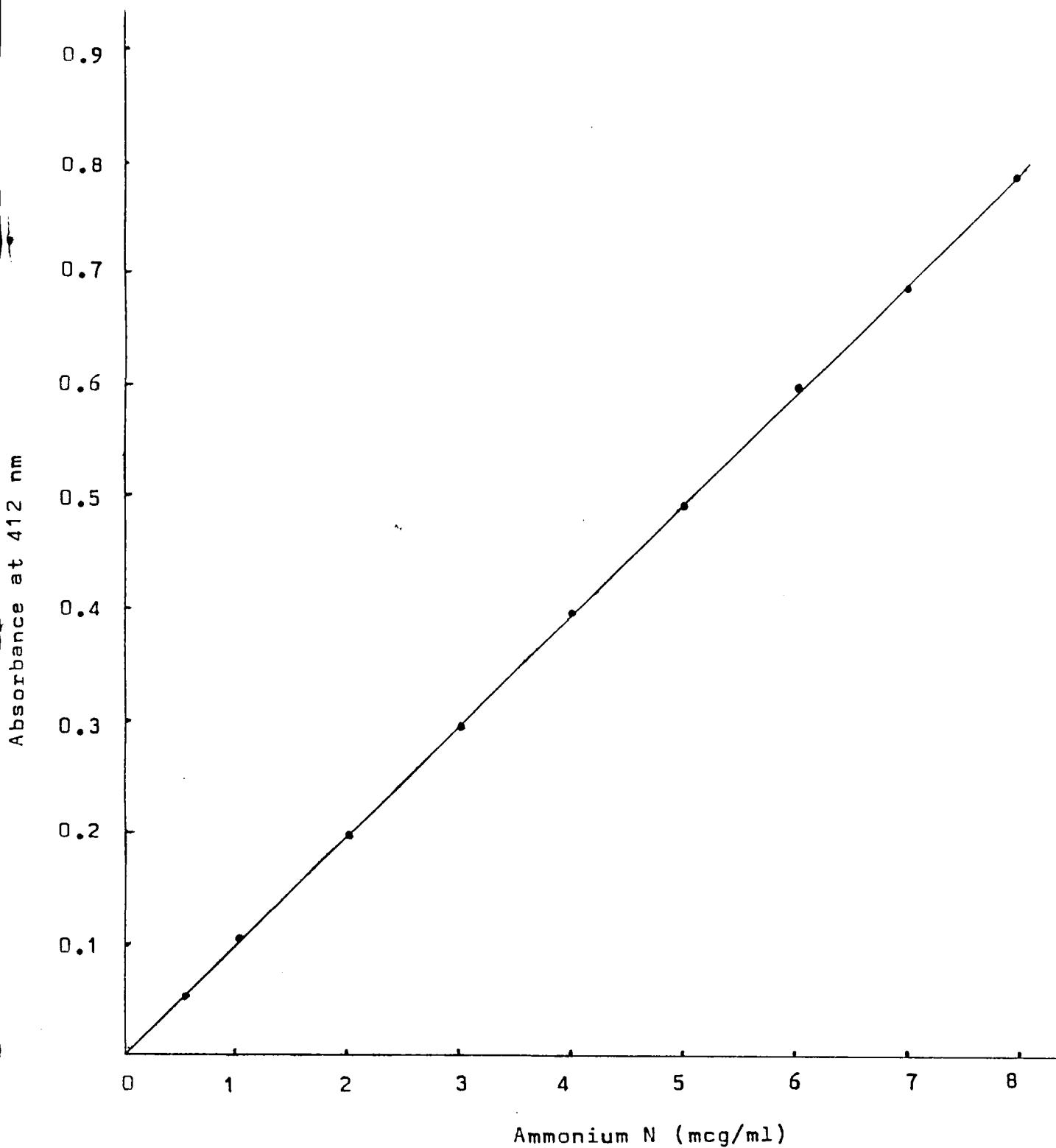


Figure 18 : Lambert-Beer's curve for Ammonium Chloride.

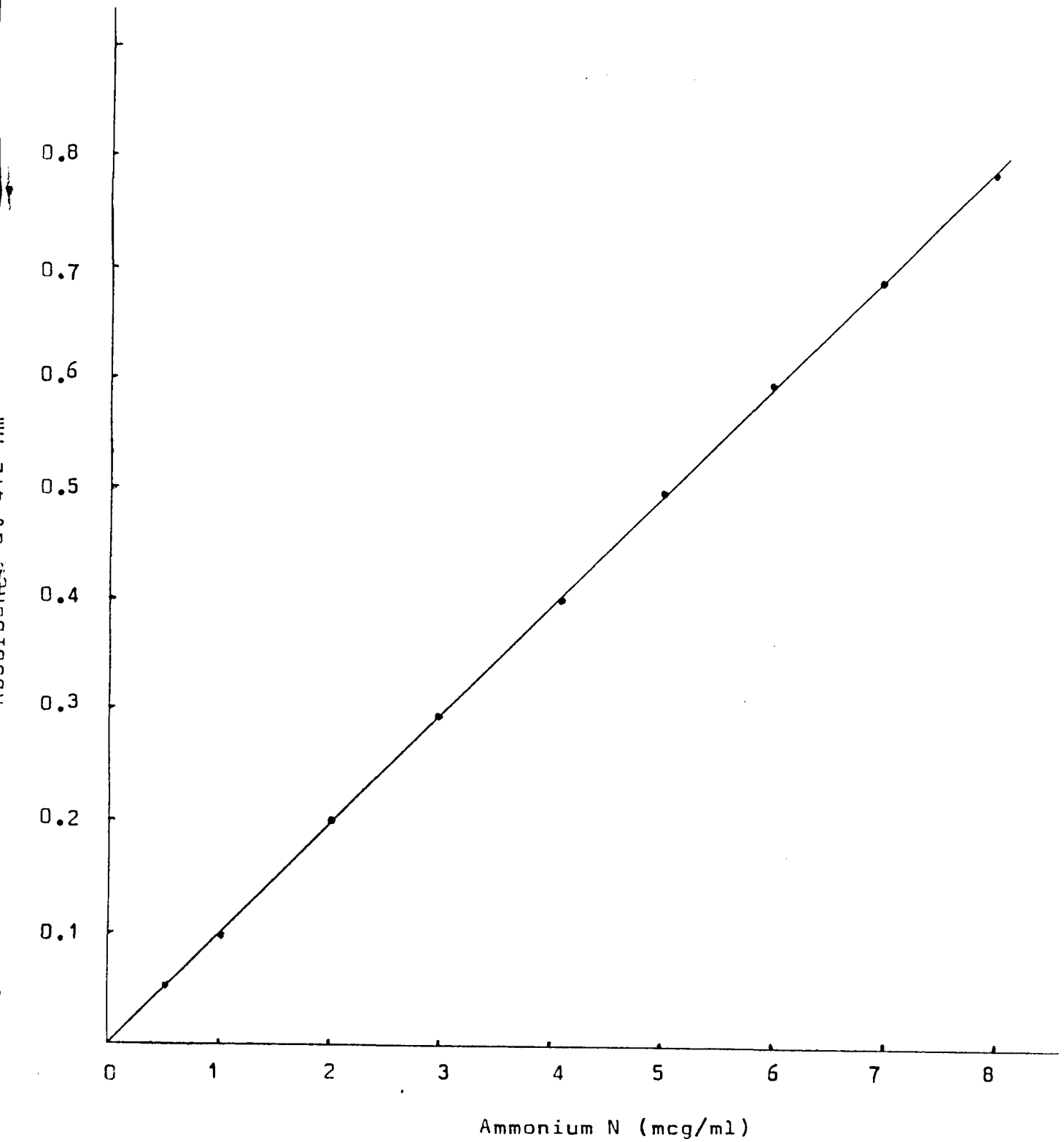


Figure 19 : Lambert-Beer's curve for Diammonium Phosphate

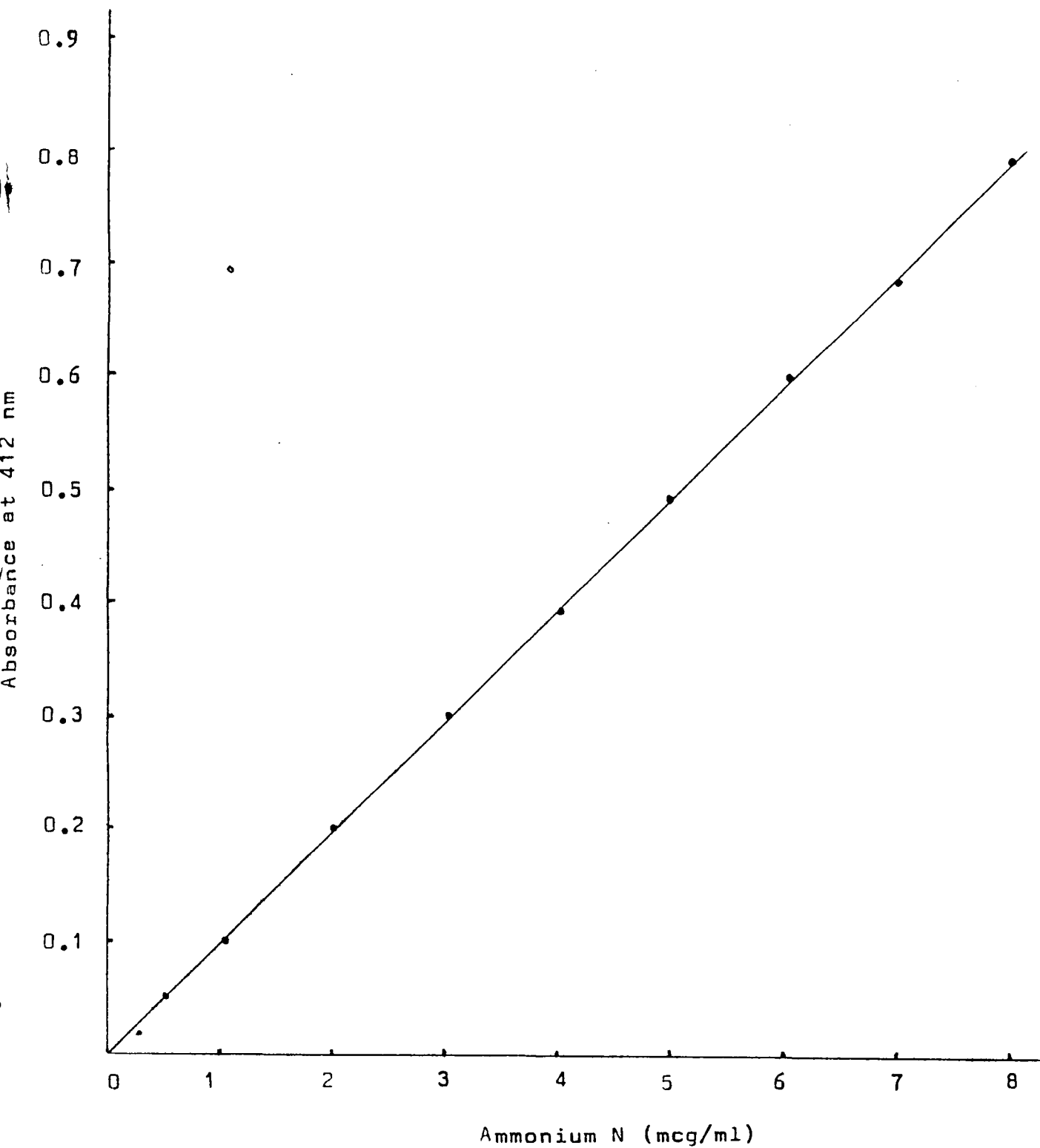


Figure 20 : Lambert-Beer's curve for Ammonium Nitrate.

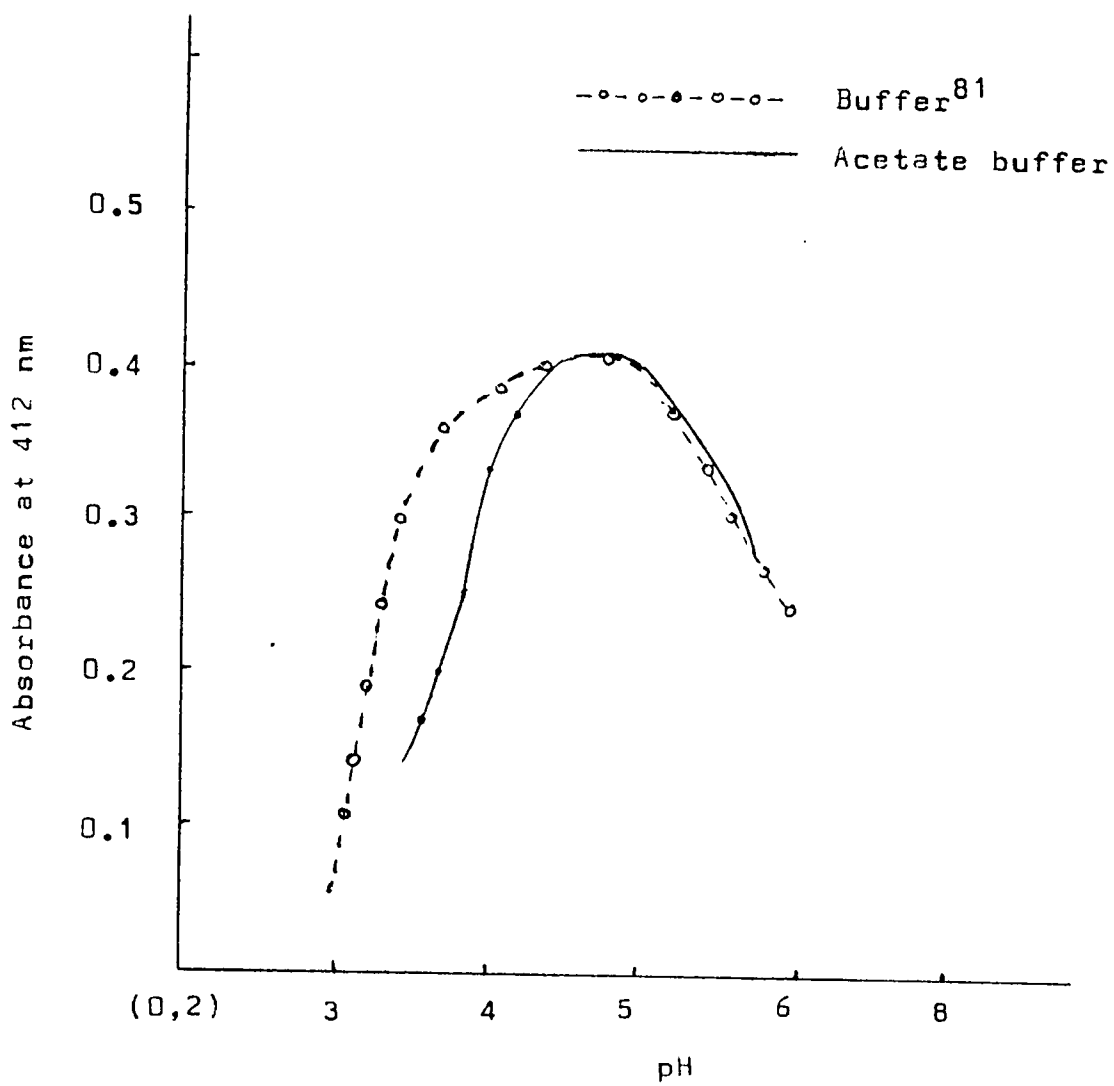


Figure 21 : Effect of pH on color intensity.

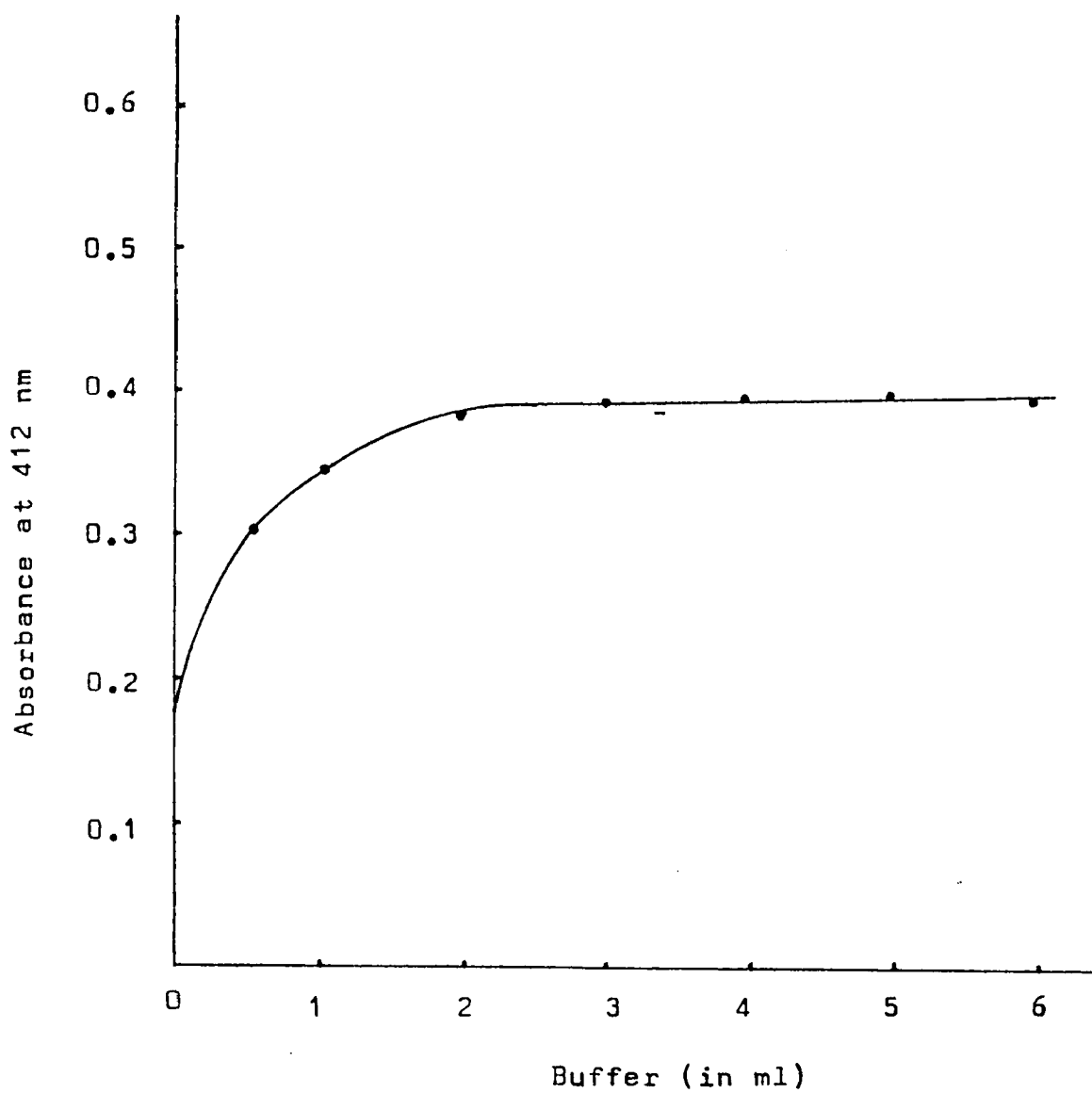


Figure 22 : Effect of buffer (pH 4.7) concentration on color intensity.

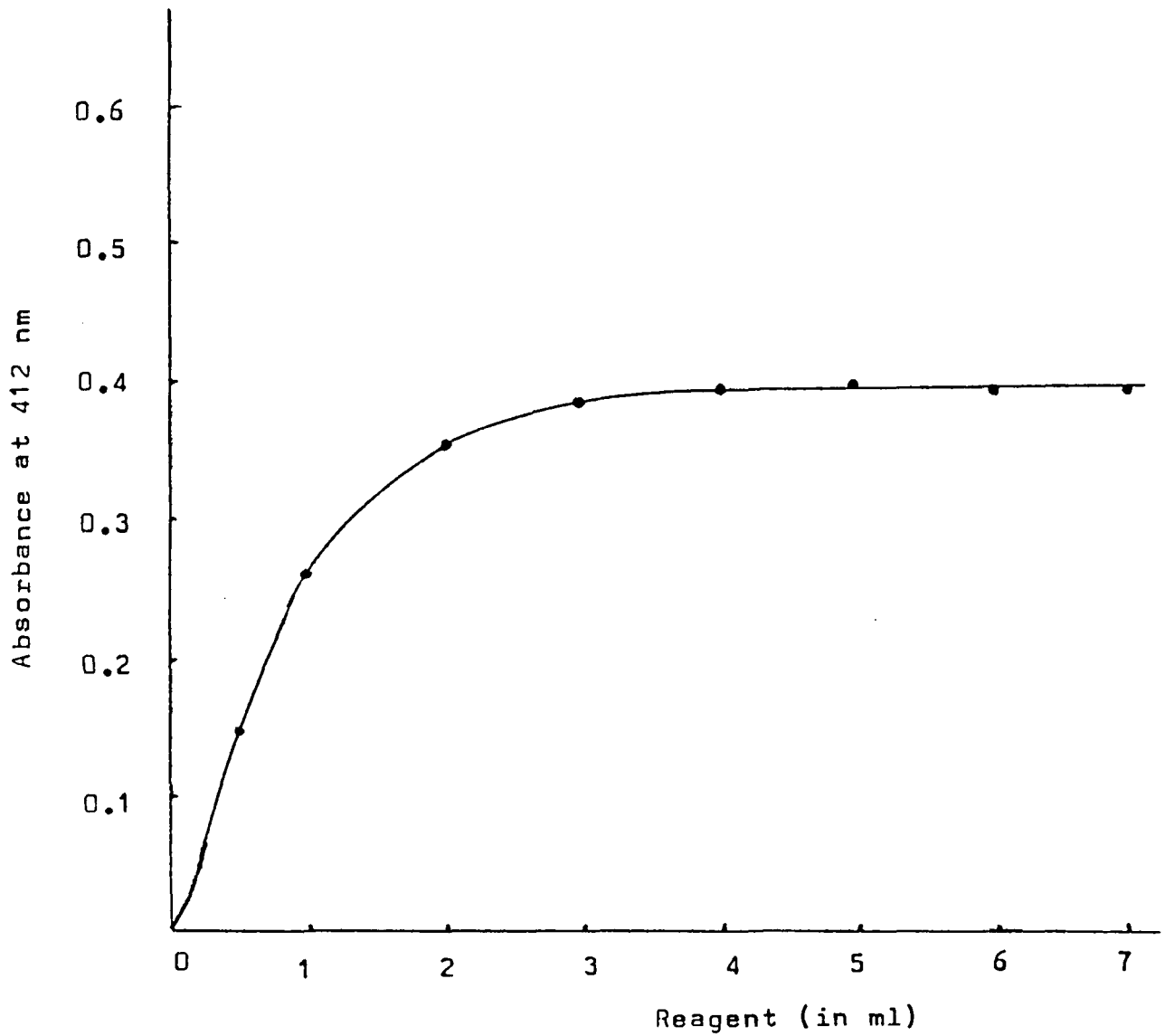


Figure 23 : Effect of reagent concentration on color intensity

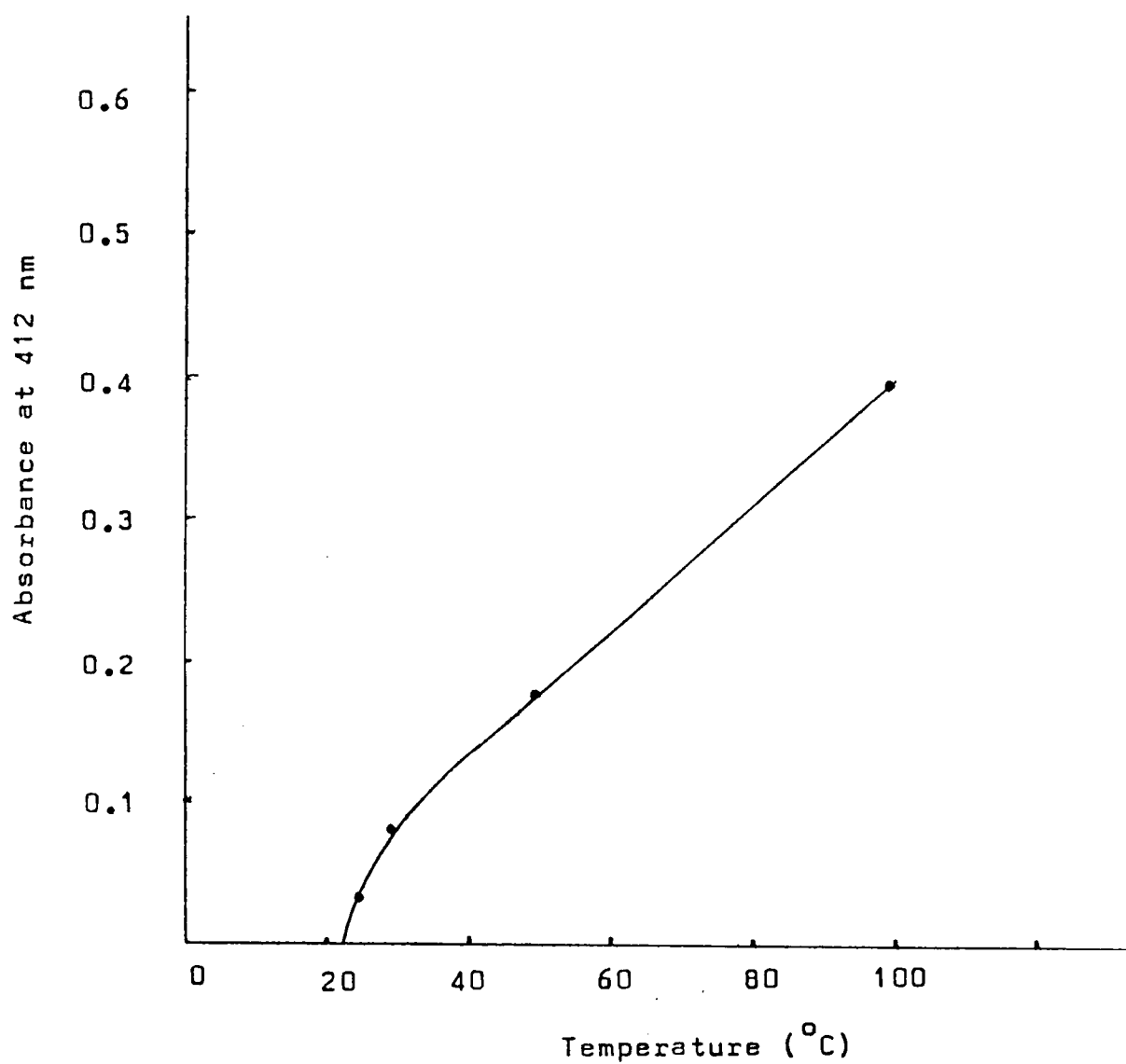


Figure 24 : Effect of temperature on color intensity.

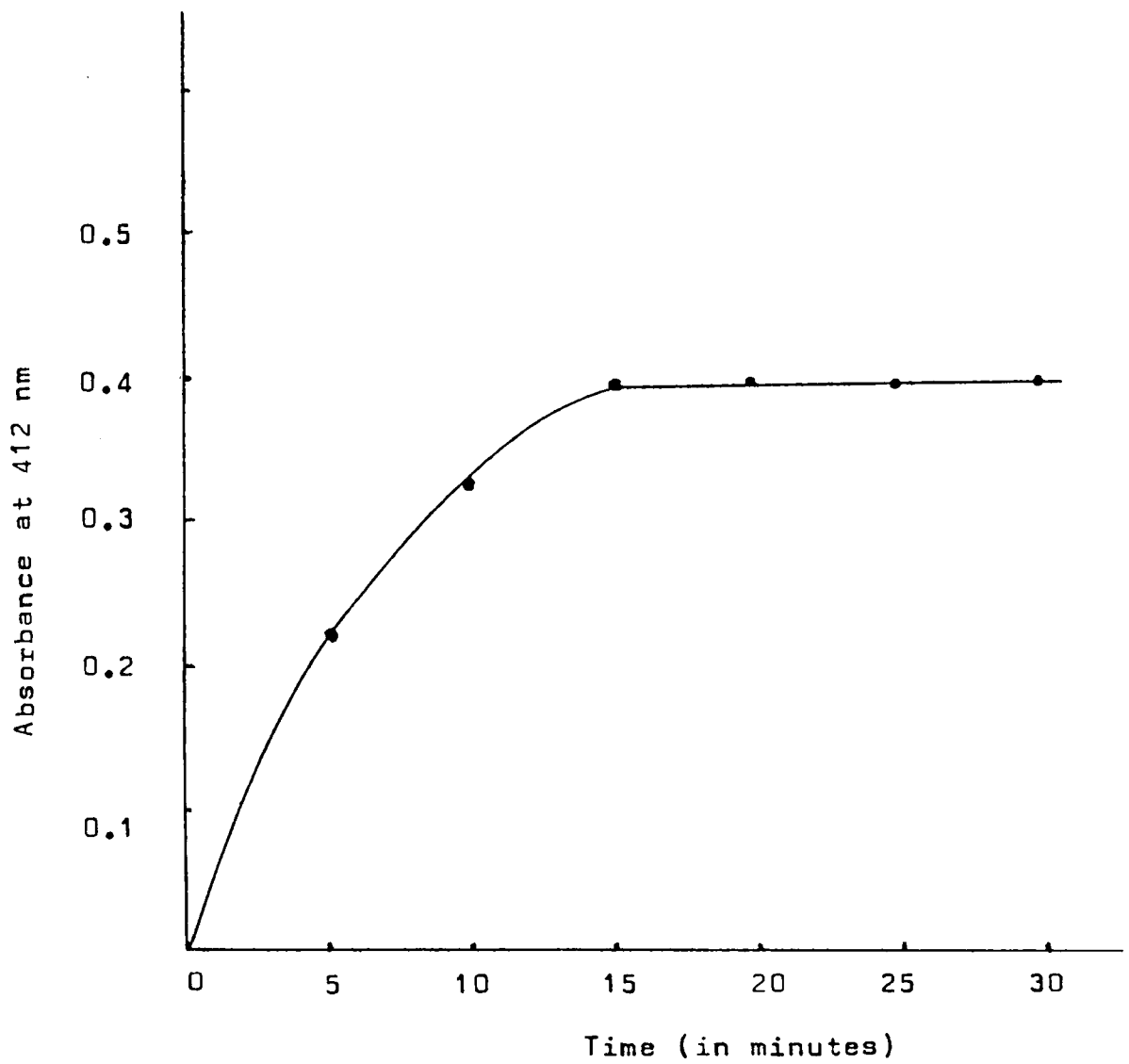


Figure 25 : Effect of heating time on color intensity.

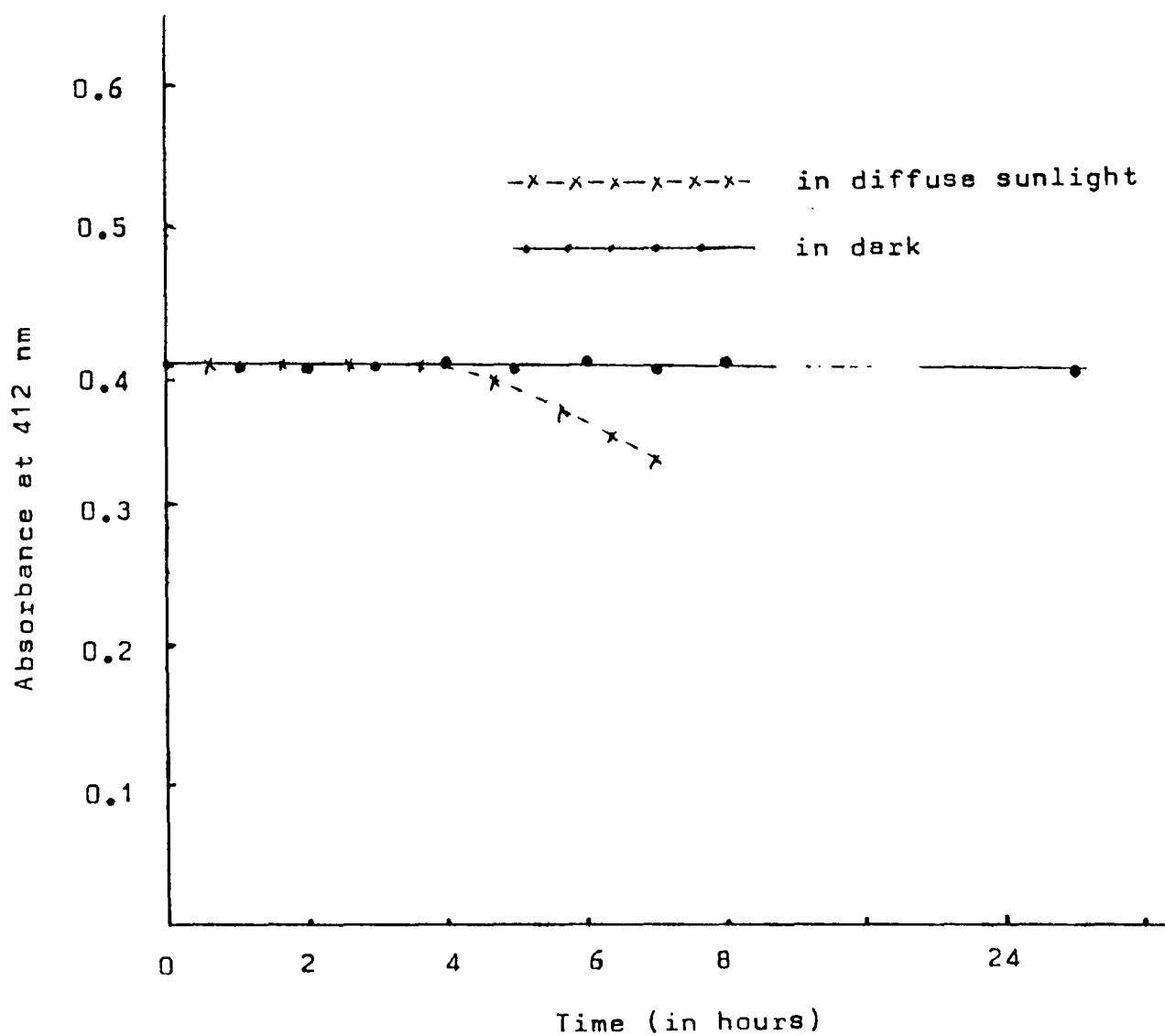


Figure 26 : Effect of time on stability of colored product.

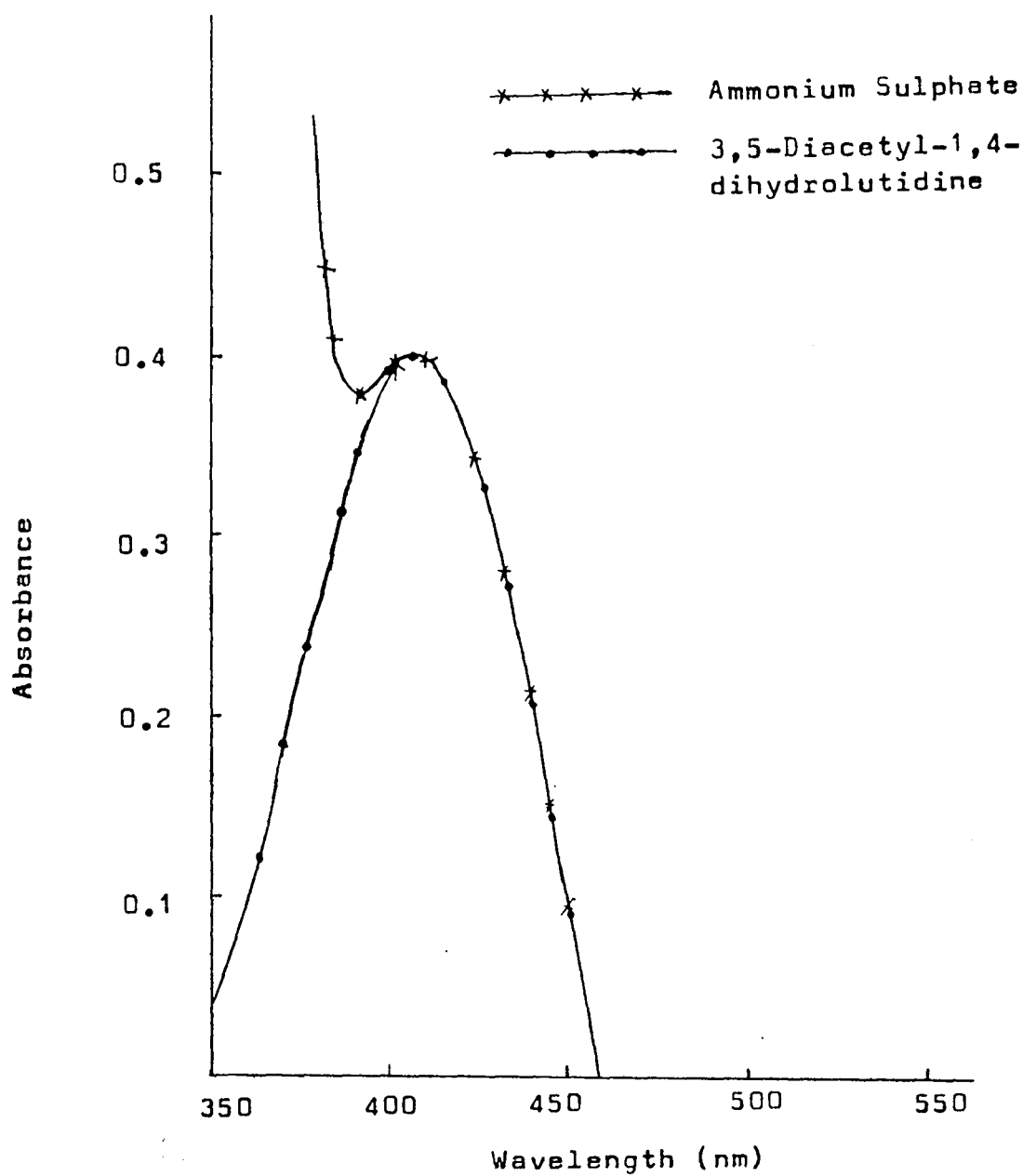


Figure 27 : Comparision of visible spectrum of colored product obtained on racting Ammonium Sulphate with reagent, with 3,5-diacetyl-1,4-dihydrolutidine.

TABLE IV

Analysis of Ammonium Nitrogen

No.	% N Recovery by	
	Proposed method	Official method ¹³⁶
1	101.00	101.40
2	100.00	99.20
3	99.00	98.60
4	99.60	99.60
5	98.60	99.80
6	100.20	101.00
7	100.40	100.20
8	99.40	100.60
9	99.80	99.60
10	100.20	99.00
	AV 99.82	99.90
	SD ± 0.713	± 0.896
	RSD $\pm 0.715 \%$	$\pm 0.897 \%$



TABLE V

Analysis of Ammonium Salts

Name of compound	% Recovery \pm SD a) by	
	Proposed method	Official method ^{136,152}
1. Ammonium Sulphate	99.98 \pm 0.70	99.99 \pm 0.95
2. Ammonium Chloride	100.00 \pm 0.71	99.91 \pm 0.88
3. Ammonium Bicarbonate	100.78 \pm 0.98	99.98 \pm 0.99
4. Ammonium Nitrate	99.93 \pm 0.99	99.95 \pm 1.10
5. Diammonium Phosphate	100.52 \pm 0.75	100.12 \pm 0.80
6. Ammonium Acetate	96.98 \pm 1.03	96.80 \pm 1.21

a) Standard deviation was calculated from the results of nine determinations.

TABLE VI

Analysis of Ammonia and its formulations

No.	Name of compound.	% Found ^{a)} by		Reference
		Proposed method	Official method	
1.	Strong solution of Ammonia	28.82 w/w	28.57 w/w	152
2.	Dilute Solution of Ammonia	9.84 w/w	9.69 w/w	152
3.	Strong Solution of Ammonium Acetate	58.80 w/v	58.05 w/v	152
4.	Ammonium sulphate in Ichthammol			
	(a) Pharmacopoeial	12.59 w/w	12.58 w/w	153
	(b) Commercial-A	-	13.54 w/w	153
	(c) Commercial-B	-	14.05 w/w	153

a) Average value of five determinations.

TABLE VII

Analysis of Ammonium nitrogen in Diammonium phosphate
in presence of urea in synthetic mixture

	Diammonium phosphate (in mg)	Urea (in mg)	% Recovery ^{a)} of ammonium nitrogen by proposed method
1	472	50	100.40
2	480	100	100.15
3	470	200	100.42
4	460	300	100.25

a) Average value of five determinations.